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Abstract:

Plants and their products, e.g. essential oils (= EOs) are mostly considered as safe medicine products. Often people do not think about the possibility that plant constituents can be very toxic and that it is wrong to generalize the fact that herbal agents are less toxic than synthesized chemical ones.

A lot of EOs, even from well known genus, operate quite toxic and harmful on the human body depending on its components. Even different species from one genus can possess various toxicity levels, for example the *Achillea* genus. Lots of EOs show similar properties: not only their toxic mode of action, but also their antimicrobial, anti-oxidative, anti-nociceptive activity, the inhibition or induction of endogenous enzymes as well as cyto- and genotoxic effects do not appear rarely.

Therefore, of that the first priority is to evaluate the composition and the EOs' properties and only afterwards its safety can be assessed.

Abstract:

Pflanzen und Pflanzenprodukte, wie zum Beispiel ätherische Öle, werden meist als sehr sichere Medikamente angesehen. Oft wird nicht darüber nachgedacht, dass die Inhaltsstoffe einer Pflanze sehr toxisch sein können und man nicht verallgemeinert sagen kann, dass sie sicherer sind als chemisch synthetisierte Wirkstoffe.
Viele ätherische Öle, auch von bekannten Pflanzengattungen, haben durchaus toxische und schädliche Effekte auf den Körper entsprechend ihrer Inhaltsstoffe. Auch verschiedene Spezies einer Gattung können unterschiedlich gefährlich sein, ein Beispiel dafür ist die Gattung *Achillea.* Viele toxische ätherische Öle zeigen ähnliche Eigenschaften auf. Neben ihrer toxischen Wirkweise, zeigen sie oft zusätzlich antimikrobielle, antioxidative und schmerzlindernde Aktivität, können wichtige endogene Enzyme hemmen oder induzieren, aber auch cyto- und genotoxische Effekte sind keine Seltenheit. Aus diesem Grund liegt die höchste Priorität darin, sich die Inhaltsstoffe und Eigenschaften eines ätherischen Öls genauer anzusehen und erst anschließend dessen Sicherheit zu beurteilen.

Index

A) Introduction:

This survey deals with toxic EOs, their composition and properties, how they can be isolated, analyzed and used. There exist many different plant genera which yield different species with toxic constituents. It is very interesting that EOs from such species are already used in ethnic medicine especially in developing countries and that they operate as important pharmaceutical agents, because of their diverse activities. The majority of the EOs in this overview possess have similar properties such as antioxidant or antimicrobial activities which probably depend on their similar composition. Same components can be found in different plants of various genera but in completely different concentrations. This is another very important point to be approached in this study, namely the dosage. Not only the toxicity, but all of the EOs' properties are depending on the concentration. This seems to be clear but nevertheless in some examples of EOs which can show converse effects on humans and animals have separately been brought forward.
In the first part of this paper it starts with a short general explanation about the assays which were used in the studies, why and how they were performed. Then, the main part of one the plants, their EOs and the acquired insight about their properties are discussed.

B) Assay methods:

1. **Isolation:** First of all, the EO has to be extracted from the fresh plant. The most common method therefore is **distillation** (for example hydro- or steam-distillation). The goal is the extraction of a scented EO by a 2.5-3h long hydro-distillation. For the subsequent separation low boiling organic solvents are used. Finally the EO can be dried over anhydrous sodium sulfate and then analyzed. [1] [2]

2. **GC (Gas Chromatography)/GC-MS(GC-Mass Spectrometry)/GC-FID:** After isolating the EO, its composition can be identified and quantified by gas-chromatography. The EO can be analyzed in a silica capillary column at a temperature from 70° to 290° C with helium as the carrier gas. After the separation by GC, the results of the GC/MS were compared with authentic standards and so mostly the compounds identified. Only when the constituents are known it is possible to decide which experiments should be performed afterwards. [1]

3. **NMR/IR/UV/MPLC (Medium Pressure Liquid Chromatography):** Used for the structural clarification which can be revealing as to the EOs properties. [1]

4. **Acute toxicity test:** The LD_{50} (median lethal dose) values have to be defined for the evaluation if the EO is dangerous, how harmful it is and which doses can be used fearlessly. Therefore, different kinds of *in vivo* tests exist: [1]
    - Method of Spearman-Karber (injection of graded doses of the oil to mice in different test groups and observation of the time of death). [1]
    - Brine shrimp (*Artemia salina*) lethality (best method for the toxicity-screening of plants). First of all 1 liter of artificial sea water has to be prepared for the *Artemia salina* cysts for hatching. Afterwards the brine shrimps are added to Petri dishes which already contain the EO in different concentrations. Finally, the mortality is evaluated after 24, 48 and 72h. [3]

5. **Subacute toxicity assay:** The animals receive daily, during a defined period of time, a dose of the EO. Afterwards the organs, blood and its hematological/biochemical parameters are analyzed. [4]

6. **Contact toxicity assay:** For this single-aged insects are needed. Because of that a glass jar has to be filled with grains and afterwards insects of both genders have to be added to the jar and incubated for 48h. Than they have to be removed and the jar with the grains needs to be incubated at 27° C for 45 days. Now the single-aged insects can be used for the contact toxicity assay. Acetone-dilutions of different EO concentrations were used and applied topically to the insects with a micro-applicator. Afterwards the insects were transferred to wheat-filled tubes and incubated at the same temperature as before. The mortality was evaluated every 24h for 3 days. [5]

7. **Fumigant insecticidal toxicity assay:** The procedure here is the same as for the contact toxicity assay with the difference that the EO-dilutions were not applied to the insects, but impregnated filter paper discs were added to the tubes. Than the incubation was performed at 25° C and the mortality was evaluated as usual. [5]

8. **Anti-nociceptive activity test:** There are different possibilities how one can assay the anti-nociceptive activity of a substance:

    - Acetic acid induced writhing test: Before the acetic acid injection, the mice are treated (injection) with the testing EO as well as its compounds. Following by the determination of the writhes number and finally the inhibition (in %) of the writhes can be calculated. [6]

    - Hot plate test (this experiment is connected to the supra spinal responses and measures the time of animal reaction to a noxious thermal stimulus): Mice are placed on a hot plate (55° C), then the EO and/or its constituents are given and afterwards the reaction-latency is evaluated. [7]

- Tail immersion test (is a spinal control and can be compared with the human flexion reflexion): This assay is performed as the hot plate test with the difference that just the tail of the animals is plunged in 50 degrees celsius hot water. [7]

- ACh (acetylcholine) induced abdominal writhing test (is a result of nicotine receptor stimulation): One hour after the injection of the testing EO, the animals get an ACh-injection and the following abdominal writhes are counted. [7]

9. **Enzyme inhibitory activity test:**
    - PKA (protein kinase A) is important for the phosphorylation in different enzymes in sugar, glycogen and lipid metabolism in the liver; it operates as well as an important enzyme in the kidney for the aquaporin 2 and urea transporter 1 exocytosis. [6]
    -
    - α-Amylase is important for keeping up the postprandial glucose levels. [6]
    -
    - AChE (Acetylcholine-esterase) [7]

10. Antioxidant capacity:

- HPTLC-PRAP (High-performance-thin-layer-chromatography-phosphomolybdenum-reducing-antioxidant-power) assay [5]

- DPPH (2,2-diphenyl-1-picryhydrazyl) assay: The measurement of the absorption takes place at the wavelength of 515nm and the used positive controls are butylated hydroxytoluene (BHT) and quercetin. [6]

- ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) assay: For the positive control the same solutions are used above at the DPPH assay, but the absorbance is measured at 734nm. [6]

- Superoxide scavenging test: The absorbance is assessed at 590nm. [6]

- β-Carotene bleaching method [8]

11. **Antimicrobial activity test:** The effect of the EOs is always tested against Gram-positive and Gram-negative microorganisms and often against fungi as well.

    - Disc and agar-well diffusion methods: The antimicrobial activity is compared with positive (for example: ampicillin, amoxicillin/clavulanic-acid, tetracycline, nystatin, augmentine) and negative control (like sterilized physiological saline solution, hexane or DMSO (dimethylsulfoxid)). Procedure: Sterile filter paper discs were impregnated with EO or the control solutions. Before the incubation (under the same conditions as described below) the discs are kept at 4° C for 2h because of the volatile EO, following by the detection of the inhibition zones. [8]

    - Determination of MIC (minimum inhibitory concentration): By means of the broth macro-dilution method different doses of the EO get tested and compared with positive (tetracycline chloride and nystatin) and negative control (DMSO in different concentrations). Procedure: Different

EO-concentrations have to be diluted with DMSO and the test-microorganisms are added to each of the dilutions as well as to the controls. Then the incubation phase at 37° C (24h for bacteria) or 28° C (5 days for fungi) begins. At the end the inhibition levels of the test samples against the microorganisms have to be evaluated. [8]

12. **Antifeedant activity assay:** It can be performed using a non-choice leaf disc method. Therefore, cabbage leaves have to be cleaned, dried and leaf discs have to be prepared. EO dilutions with acetone have to be added to each disc and after drying the insects were put to the discs for 24h or 48h. After this treatment the leaves have to be analyzed and the consumed amount of the cabbage leaf have to be calculated as well as the AFC_{50} (median antifeedant concentration) values and compared with a control (just acetone). [9]

13. **Sandwich test:** It is used for the evaluation of the pupation-inhibition. Two cabbage leaf discs were stick together, dilutions of different EO concentrations or control solutions (acetone and water) and at the end third-instar larvae were added on various discs for 48h. Then the larva were removed, fed with fresh leaves and than their weight and the pupation-number were observed. Finally the $PWIC_{50}$ (median concentration of pupae weight inhibition) and PIC_{50} (median concentration of percentage inhibition of pupation) were calculated. [9]

14. **Cytotoxicity assay:**
    - Trypan blue dye exclusion method: The cells have to be incubated with different concentrations of the EO for 3h at 37° C, afterwards they need to be washed. After coloring the cells with the stain, the amount of viable and dead cells can be identified by a light microscope. [10]

- MTT (yellow tetrazolium salt) assay: It is based on the MTT-reduction, which leads to the production of soluble blue formazan by mitochondrial enzymes. The formazan amount is adequate to the number of living cells. [10]

15. **Genotoxicity assay:**

- Comet assay (single cell gel electrophoresis): This test is used for the determination of DNA strand breaks. [10]

- DNA diffusion assay: It is used to detect the association between the amount of DNA fragments with apoptosis and necrosis. [10]

- Chromosomal aberrations (CAs) [11]

- Cytokinesis block micronucleus (MN) assay [11]

C) Toxic essential oils (plants):

1. *Achillea umbellata* (Asteraceae):

Another important species with toxic constituents of the *Achillea* genus is *A. umbellata*. As one uses *A. millefolium* against different health problems, especially in the gynecology, it is very important to know that *A. umbellata* has to be used carefully. Studies have shown that the EO of this plant is more toxic than the ones of *Artemisia absinthium*, *Tanacetum vulgare* and *Thuja occidentalis*, which contain toxic α- and β-thujones.
Different tests had been performed to show the toxic effect of *A. umbellata* in mice. [1]

Composition:

74.6% of the EO consists of fragranol (monoterpene alcohol which just can be found in the Compositae and the Tanacetum) and fragranyl acetate. These two components are most likely responsible for the intoxication signs which are shown in the pharmacological tests. The other components are fragranyl esters: formate, propanoate, 2-methylpropanoate, butanoate, 2-methylbutanoate, 3-methylbutanoate, pentanoate, hexanoate and the new corroborated fragranyl esters heptanoate, octanoate, benzoate, nonanoate, decanonate and hexadecanoate. [1]

Pharmacological tests:

For each experiment 84 adult male BALB/c mice were split into 14 groups of 6 animals. Three groups were treated with 50, 100 or 150 mg/kg *A. umbellata* EO per oral; nine groups with fragranol, fragranyl acetate and benzoate in the same dosages and the control group received olive oil. [1]

Results:

- **Acute toxicity:** The LD_{50} of *Achillea umbellata* EO was calculated by using the method of Spearman-Karber and amounts to 853 mg/kg (when injected) for mice. Compared with other *Achillea* species (*A. millefolium* 3000 mg/kg injection with no effect) or toxic EOs of another genus (for example *Artemisia. absinthium*: LD_{50} = 960 mg/kg or *T. vulgare*: LD_{50} = 1150 mg/kg) it shows a high toxicity level. During the experiment the EO injection led to death over night and the general signs of intoxication were tremors, arching and rolling, clonic convulsions, tonic extension, piloerection, sedation and hypnosis. [1]

- **Light/dark test:** The mice were put in a cage with a light and a dark room and were watched how long they would stay in each room. This test was used to assess anxiety without any aversive stimulus applied. The EO itself as well as its compounds fragranol, fragranyl acetate and

benzoate showed at the doses of 50, 100 and 150 mg/kg the same effects as 2 mg/kg dosed diazepam: A significant increase oft the time the mice spent in the light compartment was discerned. Because of that there is an assumption that the *A. umbellata* EO operates as an anxiolytic. But experts are not quite sure if this notice could also be interpreted as a paralyzing and not anxiolytic effect, because of the low locomotor activity the mice showed during the experiment. [1]

- **Anti-nociceptive activity:** The *A. umbellata* EO reduced the contraction of the abdominal muscles, as well as writhes and visceral inflammatory pain which were induced by an acetic acid injection. Nevertheless the effect is not comparable with the anti-inflammatory effect of NSAIDs (= non steroidal anti-inflammatory drugs). [1]

- **Antimicrobial activity:** The proven antimicrobial activity has been compared with other *Achillea* species and differ from each other. The reason therefore are the chemical differences. Fragranol showed the highest antimicrobial activity in *A. umbellata*. It is also a component in the most antimicrobial active *Achillea* species *A. falcata*. Disruption of cell-membrane/wall function or cell lysis caused by the EOs leads to cell death. [1]

Summary:

The study showed that the EO of *A. umbellata* has benefits but more important that it can cause death if it is used irresponsibly. It clearly belongs to the group of toxic EOs. Because of that one has to keep in mind that the dosage is the most important parameter. Further on more studies should be pursued so one can get awareness about the existing discrepancies. [1]

2. *Rosa damascena trigintipetala* (Rosaceae):

Over 150 species belong to the *Rosa* genus. One of the most important is *Rosa damascena* a sort of Damask rose which exhibits astringent, analgesic, diuretic, antioxidant, antibacterial, anti-depressant and anti-inflammatory activity.
The EO which is stored in the secretory cells, canals, cavities, epidermic cells or glandular trichomes of the plant was identified and quantified as usual by GC-MS. The identification showed 73 compounds representing four chemical categories: [2]

- **Monoterpenes** (68%): β-citronellol (17.6%), geraniol (11.4%), nerol (6.4%) and linalool (5.9%). These compounds are not only represented in the EO of *R. damascena* but also in the majority of *Rosa* species. [2]
- **Sesquiterpenes** (16.3%): β-caryophyllene, α-guaiene, α-humulene, γ-muurolene and δ-guaiene. [2]
- **Aromatic hydrocarbons:** phenylethyl alcohol [2]
- **Aliphatic hydrocarbons:** nonadecane and other alkanes and alkenes. [2]

The main constituents of all are β-citronellol, geraniol, nerol, linalool, phenylethyl alcohol and nonadecane. [2]
After identifying the composition, different experiments were performed to analyze the properties of the EOs. These tests contain the evaluation of the EOs' cytotoxic and genotoxic effects on normal human peripheral blood lymphocytes. Additionally the anticancer effects against human cancer cell lines were determined namely liver carcinoma cell line (HepG2) and breast carcinoma cell line (MCF7). [2]

Results:

- **7-AAD viability assay:** This is a common test for the estimation of the cytotoxic activities of a substance. In this case different concentrations of the rose EO were used and tested toward human peripheral blood

lymphocytes. A significant decrease in the cells functionality was shown at the concentrations >50 µg/ml, lower ones are safe. [2]

- **Determination of DNA index (**= the DNA content of tumor cells during the cell proliferation compared to that of normal cells)**:** Normal cells exhibit an DNA index of 1.02-0.96. The experiment showed the higher the EO dose is the lower the DNA index in comparison to the normal one can be found. The dead part of the cells which increases with higher EO doses is probably responsible for the low index. [2]
- **Anticancer activity:** There are four mechanisms how substances can operate against cancer cells: 1. DNA-damage, 2. DNA-synthesis-inhibition and prevention of the cell replication, 3. Mitosis-stop and 4. the cancer cell-apoptosis. Many studies approve anticancer activities of compounds which can be found in the rose EO like monoterpenes, sesquiterpenes and aromatic essential oil compounds (limonene, geraniol, farnesol, β-citronellol, menthol, β-caryophyllene, linalool, α-cadinol, eugenol). This is the reason why the *R. damascena* EO was examined for its anticancer activity against HepG2 and MCF7. Different oil concentrations were added to the samples and then tested with an ELIZER plate reader. The calculated IC_{50} values amount to 13.03 µg/ml for HepG2 and 16.44 µg/ml for MCF7. After the American National Cancer Institute substances with IC_{50} values < 20 µg/ml are considered as promising which confirms anticancer activity of the EO of rose. [2]

Summary:

When one thinks of rose products, mostly perfumes, cosmetics and foods came in mind but the compounds of *R. damascena* and a lot of other *Rosa* species have more important properties. The EO can be used in the medicine and showed great results in different assays. Probably the most important quality is the anticancer activity. Nevertheless, there are not only benefits. The dose is

the most considerable parameter, overdosed the EO leads to toxicity and decreased cell viability. [2]

3. *Anthemis segetalis* (Asteraceae):

Anthemis segetalis is one of many Asteraceae-species which can be mistaken as another plant of this family like *Matricaria chamomilla* or *Anthemis nobilis*. This is very dangerous because of the toxic constituents the plant consists of. A mix-up can lead to signs of intoxication and death especially because of the lacking data of *A. segetalis*. [3]
The complex EO consists of more than 150 different constituents, most of all: esters of allylmethoxyphenol, isomeric pentanoic and 2-pentenoic acids, especially eugenyl angelate (0.21mg/100g fresh plant), 2-methylbutanoate (0.22mg/100g) and 3-methylbutanoate (0.13mg/100g). Because of their low quantity in the oil (0.1-0.2%), the compounds had to be synthesized so they and their chemical structure could be analyzed as well as the evaluation of acute toxicity, cytotoxicity, AChE inhibition and antibacterial activity. [3]
The differences between the biological activities of the esters are probably based on the various chemical structures like different distances between the pharmacophoric points (= methoxy groups and double bounds), as well as esterification of the phenolic OH-group (lipophilicity increase). Furthermore, the position (α or β) of the methyl group is responsible for the acute toxicity (lower in β-position) as well as the cytotoxicity (lower in γ-position). [3]

Results:

- **Acute toxicity:** As always there are a lot of components which show no toxic activity when given in a certain concentration, but also ones which are moderate toxic. The toxicity level of the harmful constituents are comparable and the LC_{40} value amounts from 0.12 to 0.18 mmol/ml. [3]

Compound	% Mortality (24h)	% Mortality (48h)	LD_{40} mmol/ml (24h)	LD_{40} mmol/ml (48h)
Eugenol	55.0	82.5	0.15	0.05
2-Allyl-3-methoxyphenol	72.5	82.5	0.12	0.06
2-methylbutanoate	62.5	92.5	0.12	<0.02
3-methylbutanoate	55.0	80.0	0.16	0.04
senecioate	42.5	70.0	0.18	0.07

Table 1: Acute toxicity of *A. segetalis* constituents (adapted and newly drawn from [3])

One can see the mortality effect of the individual components is very high especially after 48h. But the EO itself is not as toxic as these constituents because of its complex composition of dangerous and harmless elements. [3]

- **Cytotoxicity (cell anti-proliferative activity):** In a concentration of 5mg/ml all tested components showed cytotoxic effects against non-transformed MRC5 (human lung fibroblast) and transformed A375 (melanoma) cell lines. The lowest anti-proliferative effect with 60-70% inhibition showed 3-methylbutanoate and 4-allyl-3-methoxyphenyl tiglate. It is also known that the effect depends on the dosage. The highest cytotoxic effect exhibited 2-methylbutanoate, 4-allyl-2-methoxyphenyl angelate and 2-allyl-3-methoxyphenol with 50-60% inhibition of fibroblasts and 20-40% of melanoma cell line in a concentration of 50 µg/ml. [3]

- **Enzyme inhibition (acetylcholinesterase):** An AChE inhibition was only shown by eugenic esters of angelic (35.4%), scencioic (17.4%) and 2-methylbutanoic acids (26.1%). [3]

- **Antibacterial activity:** In the antibacterial activity test against 17 bacterial strains (Gram-positive and Gram-negative have similar sensitivity) the tested components showed moderate effects (MIC = 0.32-6 mg/ml). Especially 2-allyl-3-methoxyphenol (MIC = 0.32–3.00 mg/ml), 2-allyl-5-methoxyphenyl tiglate (MIC 0.32–6.00 mg/ml) and eugenol (0.32-1,5 mg/ml) are highly active against a broad spectrum of microorganisms. They operate by damaging the bacterial cell wall and membrane. *Proteus mirabilis* (an isolate from human urine) and *Enterobacter cloacae* (a wound isolate) are very susceptible whereas the highest resistance was shown by *Sarcina lutea*. [3]

Summary:
As the *A. segetalis* EO shows great effects on different tumor cell lines and solid antimicrobial activity, it could be seen as a potential therapeutic agent. But more studies about its properties and safety have to be performed before it can be used fearlessly. [3]

4. *Cupressus lusitanica* (Cupressaceae):

The EO of the leaves of *C. lusitanica*, native in Cameroon, is an interesting phytotherapeutic agent. Many important therapeutic properties are attributed to this plants' EO like antibacterial/-fungal/-viral/-microbial, antioxidant, anticancer, sedative, anti-inflammatory, analgesic, spasmolytic, and local anesthetic properties for the treatment of hemorrhoids, rheumatism, whooping cough and styptic problems as well as the inhibition against dermatophytes. [4]

The identification of the leaf essential EO yielded 49 components, mainly represented by germacrene D (18.5%) epi-zonarene (8.2%), ciscalamenene (8.2%), terpinen-4-ol (6,3%), linalool (6.0%) and umbellulone (6.0%). Self-

evidently the composition can vary between the EOs of *C. lusitanica* with different origins. [4]

Results:

- **Acute toxicity:** The animals (male Wistar albino rats and Swiss mice) showed after a single oral dose (4 g/kg or more) of the EO uncontrolled movements and an increase of their respiratory rhythm as well as deaths of the mice (most likely because of the respiratory failure) within 48 hours. The calculated lethal dose was 6.33 g/kg which means that the EO can be classified as little toxic. [4]

- **Subacute toxicity:** The rats' response, after a four week treatment with the EO, were movement- and aggressiveness-decrease, touch and noise sensitivity and increased respiratory rhythm. Deaths could not be noted, but very well the decrease of many important blood- and serum-components like red and white blood cells (at concentrations of 1.5-2 g/kg), haematocrit, creatinine and protein levels. Mostly affected by the changes in the protein levels are the liver, lungs, spleen, serum and especially the heart and kidneys which already showed a decrease at the dose of 0,8 g/kg. These changes could be attributed to tissue injuries and lead to metabolic, physiological variations and behavioral problems. Reasons for the blood cells reduction could be affections of liver and spleen or bone marrow failure. Furthermore, the daily EO intake affected the transaminase levels (AST, ALT) which are existent mainly in the liver and an increase is mostly a sign of liver damage. The EO operated cytotoxic against the liver by affecting the permeability of the cell membrane. The higher the used EO concentrations were the stronger effects on the animals organism could be noticed. [4]

- **Antimicrobial activity:** The activity of the EO depends on the chemical structure of the constituents. β-myrcene, limonene, phellandrene, γ-

terpinene, caryophyllene, and thymol probably achieves the highest effects. The antimicrobial properties were tested using two methods against 8 bacteria (for example *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*) and 6 fungi (for example *Candida albicans*, *C. lusitaniae* and *C. tropicalis*). Moderate antimicrobial activity was shown against all microorganism, but bacteria (equal susceptibility between Gram-positive and Gram-negative bacteria, but resistances are more common in Gram positive bacteria) reacted more sensitive in the disc diffusion method (inhibition zones: bacteria = 11-18 mm, fungi = 7-14 mm) and fungi in the macro dilution test (bacteria MIC = 1.25-10%, fungi MIC = 0.16-1.25%). The hydrophobicity of the EO and its constituents is beside the chemical structure, responsible for the antimicrobial effects. It leads to the partition of the cell membrane- and mitochondrial-lipids and for the cell structure distribution. [4]

Summary:

Even tough the EO of *C. lusitanica* is classified as little toxic, the effects it caused in mice and rats showed that in high doses and constant intake it operates toxically and leads to a lot of damages in different parts of the body. Because of that one should be aware of its possible dangerous properties. [4]

5. *Tanacetum macrophyllum* (Asteraceae):

As the usage of synthetic chemical insecticides is forbidden in a lot of countries because of their harmful effects on the humans health. More and more studies - especially with EO plants - are performed to evaluate their antimicrobial activity. The results of these investigations are paeromising depending on the used plant genus. Good results were achieved in the studies with the *Tanacetum* species. They showed different interesting and useful

properties because of their anticancer, antifibrinolytic, anticoagulant and herbicidal activities. [5]

Herein the focus lies on the EO of the leaves and the flowers of *T. macrophyllum* native in Turkey. As always the composition varies between the oils of plants from different geographical origin. [5]

- **Flower EO:** 101 components were identified in the EO of the flowers of which 64.9% were oxygenated sesquiterpenes with γ-eudesmol (21.5%), sesquilavandulol (20.3%) and copaborneol (8.5%) as its main constituents. [5]

- **Leaf EO:** Compared with the flower EO less components were identified in the oil of the leaves - 79 in total. The dominant substance groups are here oxygenated monoterpenes (47.2%) and sesquiterpenes (24.9%). The major constituents differ from the ones of the flower EOs and contain copaborneol (14.1%), 1,8-cineole (11%), bornyl acetate (9.6%) and borneol (6.3%). [5]

Results:

- **Contact toxicity:** The toxicity assays were performed against *Sitophilus granarius* and showed high toxicity levels as the mortality was 88.93% for the leaf EO and 50% for the flower EO at a dose of 0.2 µL/mL. As 1,8-cineole and camphor are well known for their toxic activity against *S. granarius* and the leaf EOs main constituents are 1,8-cineole as well as bornyl acetate and borneol (which exhibit lots of similarities to camphor), these components are most likely the reason for the high toxic potential of the oil. This statement can be confirmed as the flower EO does not contain these constituents and because of that it shows much less contact toxicity. [5]

- **Fumigant toxicity:** Neither the EO of the leaves nor the one of the flowers showed any significant fumigant toxicity against *S. granarius*. The reason for that could be the high boiling points of the main constituent which would explain the low volatility of the compounds and that is why the oil did not affect the insects at all. [5]

- **Antioxidant activity:** The radical scavenging activity was evaluated by the DPPH-scavenging activity and the HPTLC-PRAP assays. At the concentration of 10 mg/mL the leaf EO possessed the highest measured antioxidant activity of 47.7% an the flower oil of 29%. Nevertheless, these effects are not comparable with the antioxidant properties of the positive control α-tocopherol (94.5%). [5]

After finding many dissimilarities in the chemical composition of different *T. macrophyllum* EOs, it is clear that various chemotypes of the oil exist. This variety - especially in the amount of the constituents and the different main components - changes the properties of the EOs. Geographical-, climate-, ecological-differences as well as various methods and instruments or EOs of different plant-parts could be the reason of the existence of chemotypes. [5]

There are three chemotypes:

- β-Eudesmol-chemotype
- γ-Eudesmol/sesquilavandulol-chemotype
- δ-Cadinene-chemotype [5]

Summary:

T. macrophyllum EOs showed that their properties can be compared with the other antimicrobial operating *Tanacetum* species. They are another proof that natural products can sometimes be enough effective but much safer than synthetic insecticides in pest control. More studies should be performed to

confirm this statement and to evaluate the toxic effects of the EO against humans. [5]

6. *Artemisia annua* (Asteraceae):

Artemisia annua is mostly identified by its component artemisinin (endoperoxide sesquiterpene lactone) which is used in the treatment of malaria, but there are a lot of other constituents with barley known biological and pharmacological activities. The EO of the Serbian species consists of 58 components and beside artemisinin the most important ones are artemisia ketone (35.7%), α-pinene (16.5%) and 1,8-cineole (5.5%). Another reason why there is few information about this plant is that there exist 5 different chemotypes which show various chemical and volatile profiles and this leads to varied activities. Especially the metabolites vary between the populations. But the biggest differences show species with various geographic origin. [6] Because of the incomplete toxicity data on *A. annua*, experiments were performed including testing the anti-nociceptive, antimicrobial, antioxidant, enzyme inhibiting potential and *in vivo* and *in vitro* acute toxicity tests. Therefore, aboveground parts (leaves, stems, flowers, fruits) of the plant were extracted by hydrodistillation, analyzed by GC-MS/GC-FID and tested. [6]

In vitro assay results:

- **Antioxidant capacity:** Many diseases lead to oxidative stress which is the reason why it is relevant to analyze if the EO shows a high antioxidant activity which depends probably on the flavonoid concentration and the presence of aromatic components in the EO. The analysis showed that the EO itself as well as artemisia ketone possesses the lowest antioxidant capacity. Nevertheless, the *A. annua* EO and its components have a higher potential than the controls, even though they possess low antioxidant properties. [6]

- **Enzyme inhibiting activity:** The EO and its constituents barley inhibit PKA (about 1%) which is very good because of the important assignments this enzyme possesses in the liver and kidney. Equally the α-amylase inhibition by the EO and its components is not existent which means that it is not able to cause complications for diabetics. [6]

- **Antimicrobial activity:** The essential oil *A. annua* inhibits the growth of Gram-positive (highest susceptibility) and Gram-negative bacteria as well as fungal strains (highest resistance). Its MIC (minimal inhibitory concentration) averages 2.5-20 mg/ml whereas the most sensitive microorganisms are *S. lutea* and *Aspergillus fumigatus*. However artemisia ketone possesses the greatest antimicrobial activity with MIC = 0.07-10 mg/ml followed by 1,8-Cineole while α-pinene shows the weakest antimicrobial effect. Nevertheless, the showed antimicrobial activity by the EO and its components is not enough to be used as oral/ systemic antimicrobial pharmaceuticals. [6]

In vivo assay results:

- **Acute toxicity:** According to the calculations the LD_{50} for mice amounts to 1350-2000 mg/kg. After 7 days the *A. annua* EO injection leads to death. The intoxication signs also showed by children and adults who took the EOs were: tremors, increased motor activity, piloerection, hyperesthesia, irregular breathing and sedation as well as tonico-clonic or solely clonic convulsions. There are big differences between the LD_{50} values of the constituents. The lowest value possesses α-pinene with 500 mg/kg therefore it is responsible for the EOs toxicity. The reason for the differences in *A. annua* samples are their various chemical compositions. [6]

- **Anti-nociceptive activity:** The anti-nociceptive activity of *A. annua* is based on two mechanisms: desensitization of TRPV1 (TRPV1

activation leads to abdominal pain) and blocking of TRP 1. It has been confirmed as the acid-induced writhing was reduced by camphor (64%), 1,8-cineole (54%) and α-pinene (39%) in a dose of 400 mg/kg. Nevertheless, the EO cannot reach the analgesic effect of NSAIDs. [6]

- **Hepatotoxicity:** Alanine-Amino-Transferase (ALT), Aspartate-Amino-Transferase (AST) and Pseudo-Choline-Esterase (PCHE) are important liver markers which change when there is a liver disorder. In the experiment the parameters did not vary when the rats were treated by the *A. annua* EO, in fact it had an protective effect against CCl_4 which is hepatotoxic. Observations showed that the damage caused by CCl_4 was reduced in rats which received additionally *A. annua* EO. Furthermore, histopathological observations showed that CCl_4 causes extensive injuries like hepatocellular degeneration and necrosis around the central vein in the liver, but the hepatoprotective effect of the EO in this case are non existent. [6]

- **Nephrotoxicity:** Important kidney markers are urea and creatinine serum levels. The test with CCl_4 showed here the same results as in the liver. A mild nephroprotective effect could be detected as the his-pathological lesions decrease under the influence of the EO. [6]

Summary:

Experiments showed that the *A. annua* EO possesses nephroprotective, antimicrobial and low antioxidant effects. Compared with other EOs it is not very toxic, therefore it does not represent a health risk. However it is very important to know that the volatile profile of *A. annua* varies especially in plants with variable origin which means that different EO samples can have various biological and pharmacological characteristics. [6]

7. *Achillea falcata* (Asteraceae):

Different species of the *Achillea* genus are used in medicine and have possess benefits. The problem is that there is little knowledge about their toxicity and that one has to make out which species can be used fearlessly and which ones are highly dangerous. The identification showed that *A. falcata* EO, not only of the aerial as well of the underground parts, consists of 180 constituents, primary (more than 40% of the oil) of trans-sabinol and its esters (the formate, tiglate, acetate, butanoate, isobutanoate, 2-methylbutanoate and 3-methylbutanoate). Whereas the trans-sabinyl formate, tiglate, angelate and senecioate are newly identified compounds. Furthermore, it possesses sesquiterpene lactones which are cytotoxic. Additionally, there is no doubt for the existence of different chemotypes which show various biological and toxicological profiles. [7]
These compounds (sabinene, sabinol and sabinyl acetate) can also be found in the highly toxic *Antemisia. absinthium* and *Juniperus sabina. J. sabina* irritates the mucosal lining of the intestinal tract, causes occlusion of the kidneys with hematuria, congestion of other abdominal viscera, menorrhagia and abortion. Nevertheless, *A. falcata* is used against stomach ache, fever, hemorrhoids and operates as an antimicrobial, antioxidant and anti-platelet as well as protective against oxidative damage in human blood cells. [7]

Results:

- **Acute toxicity:** The toxicity level was tested by the brine shrimp assay. Moderate toxicity of trans-sabinol and some of its esters was shown in a concentration of 0.0025-0.2 mg/ml. The esters of tiglic (LD_{50} = 0.15 mmol/L after 24h) and senecioic (LD_{50} = 0.04 mmol/L) acids are the most toxic. Not only brine shrimp but also mammals are affected by the toxicity of the *A. falcata* EO. [7]

- **AChE inhibitory activity:** The structural similarities between trans-sabinyl esters and the AChE inhibitors rivastigmine and physostigmine lead to the assumption that *A. falcata* EO possesses this property as well. Here the presence of a double-bond is important for the activity which can vary because of the differences in the substitution pattern around carbon–carbon double bond. Trans-sabinyl formate has the lowest inhibitory effect whereas tiglate and senscioate show the highest activity (AChE-activity reduction up to 40%). [7]

- **Anti-nociceptive activity:** Antinociception and analgesia could be considered as signs of acute intoxication. For this purpose three experiments were performed: ACh (= acetylcholine) writhing (highest activity = trans-sabinyl acetate), hot plate (highest activity = trans-sabinyl tiglate) and tail immersion experiment. The various tests allow the evaluation of the different mechanisms of anti-nociception. Several groups received trans-sabinol and five of its esters in different doses (12.5, 25 and 50 mg/kg) and two groups got 5 mg/kg morphine. The tests showed that all compounds affect the peripheral and central nervous system and possess at least a little anti-nociceptive activity. [7]

Summary:

The experiments and their results show that the *A. falcata* EO is very potent, strong and dangerous, because of that it has to be used carefully and in low concentrations. It is one of the EOs which can harm animals and humans easily and can cause intense intoxication signs. [7]

8. *Schinus molle* (Anacardiaceae):

The *Schinus molle*, also known as pink- or American-pepper natively found in South Americas subtropical regions, leaf and fruit EO possesses different properties (antibacterial, antiviral, antiseptic, anti-fungal, anti-oxidant, anti-inflammatory, anti-tumor, anti- spasmodic, analgesic, stimulant and anti-depressant activity), because of that it has been used in folk medicine treating toothache, rheumatism, menstrual disorders, and respiratory/urinary-tract infections. [8]
As always different parts of the plant are differently compounded, consequently the EOs possess various properties, more specifically the intensity of their effects varies. Another reason regarding the different chemotypes and various composition-pattern are the origin as well as the influence of extrinsic conditions and different intra-specific effects. Because of that the composition and the results of the leaf and fruit EOs are discussed separately. [8]

Leaf EO:

The 22 identified components are mainly monoterpenes (69.3%) and sesquiterpenes (17%) including α-phellandrene (25.9%), limonene (11.7%), β-myrcene (11.1%), β-phellandrene (10.5%) and elemol (9.0%). [8]

Results:

- **Antioxidant activity:** Not only the free radical scavenging effect (4.8%), but also the lipid peroxidation inhibition (57%) were tested using two methods and the IC_{50} level (0,8 mg/ml) was calculated. The highest antioxidant capacity showed α-pinene, sabinene, limonene, β-myrcene, α- and β-phellandrene as well as the presence of oxygenated sesquiterpenes (14%). Compared with ascorbic acid which possesses great antioxidant property the effect of the EO is much lower. [8]

- **Antimicrobial activity:** A broad spectrum of microorganisms is sensitive against the *S. molle* EO especially *S. epidermidis* (inhibition zone 11 mm), *S. aureus* (10 mm), *E. faecalis* (10 mm) and *C. albicans* as well as *P. aeruginosa* and *Salmonella entiritidis serovar Typhimurium* which are both antibiotic-resistant Gram-negative bacteria. The EO is more effective against Gram-positive strains with MICs = 63 and 125 µg/ml. Furthermore, it shows anti-fungal activities against food spoilage, storage and animal pathogenic fungi most of all *Rhizophus stolonifer* (500 µg/ml), *Aspergillus japonicus* and *Rhizophus oryzae* (750 µg/ml). [8]

- **Acute toxicity:** The toxicity assay was performed by an *in vitro* (*A. salina*) and an *in vivo* test (Swiss albino female mice) and showed different effects. The in vitro experiment exhibited high toxicity with a LC_{50} level of 47 µg/ml. In the *in vivo* test the mice did not show any intoxication signs or changes in defined behavioral parameters as catalepsy, postural reflexes, sensitivity tail test, pineal reflex and motor activity. Only in doses of 1000 and 2000 mg/kg passive behavior was noticeable in the first 24h. Even after the assay the examined organs and tissues remained normal. [8]

Fruit essentail oil:

16 constituents were identified in the EO of the fruits, 98% of them are monoterpenes and only 0.5% are sesquiterpenes. The major components are β-myrcene (51.3%), limonene (14.1%), α-phellandrene (14.0%) and β-phellandrene (11.0%). [8]

Results:

- **Antioxidant activity:** The antioxidant effects of the fruit EO is lower than the ones of the leaf with values below: free radical scavenging

effect (5.5%), lipid peroxidation inhibition (19%), IC_{50} level (4.2 mg/ml) and oxygenated sesquiterpenes (0.3%). [8]

- **Antimicrobial activity:** The most potent effects are shown against *S. aureus* (inhibition zone 10 mm), *E. faecalis* (8 mm), *P. aeruginosa* and *S. entiritidis serovar Typhimurium*. The activity against Gram-positive strains is here greater as well with MICs of 125 and 500 µg/ml. The anti-fungal effects correspond to the ones of the leaf EO. [8]

- **Acute toxicity:** The toxicity studies did not exhibit any differences compared to the already discussed leaf EO. [8]

Summary:

More studies about the *S. molle* EO are planned especially in the cancer research. First it has to be tested if it is causing hepato- and nephrotoxicity only then tests about its possible effects on human tumor cells (cellular proliferation, viability and apoptosis) can be performed and evaluated if the EO exhibit therapeutic properties. [8]

9. *Chenopodium ambrosioides* (Chenopodiaceae):

Chenopodium ambrosioides is a plant native in subtropical, tropical and temperate regions of Central America. Its EO - with α-terpinene (26.8%) and p-cymene (49.6%) as its major constituents - shows insecticidal and medicinal properties. The EO is effective in the treatment of digestive, respiratory, urogenital, vascular and nervous disorders, diabetes and hypercholesterolemia and operates as a sedative, antipyretic and anti-rheumatic agent. As the diamondback moth (DBM), Plutella xylostella, develops more and more resistance against chemical pest control agents, the EO of *C. ambrosioides* could appear as an effective alternative in this matter. Because of that its

contact and fumigant toxicity against the diamondback moth were tested. [9] [12]

Results:

- **Contact toxicity:** The EO operated more toxic against the second-instar larvae (LD_{50} = 0.353 µg/larvae) then against the third-instar larvae (LD_{50} = 2.916 µg/larvae) or the fourth-instar larvae (LD_{50} = 4.843 µg/larvae). Furthermore, it possessed a higher contact toxic activity than α-terpinene (LD_{50} = 104.06 µg/larvae) and p-cymene (LD_{50} = 87.51 µg/larvae). Consequentially one can say that the synergy of the EO's constituents causes higher toxicity than its isolated components. [9]

- **Fumigant toxicity:** The results of the EO's activity were comparable with the ones which were achieved in the contact toxicity assay. Also the fact that the EO itself was more effective than the isolated major compounds could be confirmed in this assay too. The LD_{50} values of the second-, third- and fourth-instar larvae are: 2.437, 6.142 and 7.300 mg/L air. The values for α-terpinene and p-cymene against the third-instar larvae were 284.623 and 221.666 mg/L air [9]

- **Antifeedant activity:** Terpenoids, which also are important components of the *C. ambrosoides* EO, are probably responsible for the antifeedant effects. As expected, the leaf consumption decreased with increasing EO concentrations which confirms the EOs' antifeedant activity. The AFC_{50} values for the 24h- and the 48h treatment were 66.81 mg/L and 78.24 mg/L. [9]

- **Pupation inhibition:** The pupation percentage as well as the pupal weights were lower than the control in all tested concentrations, decreasing with increasing EO-doses. The $PWIC_{50}$ was 176.5 and the PIC_{50} was 111.6 mg/g leaf. [9]

- **CarE (Carboxyl-Esterase) and GSTs (Glutathione-S-Transferases) activity:** The LD_{50} dose and higher inhibited the CarE activity but induced the GSTs activities when the EO was applied topically. Fumigant treatments only affected CarE negatively and GSTs not at all at doses below the LD_{50} value. Concentrations above the LD_{50} value inhibited the GSTs as well. [9]

- **SOD (Superoxide-Dismutase), POD (Peroxidase) and CAT (Catalase) activity:** The SOD (at all concentrations) and CAT(noticeable effects only at doses above LD_{10} value) activity were induced but at all concentrations the POD activity was inhibited by the contact treatment with the EO. The difference to the contact treatment is that the fumigant exposure caused the same effects on all three enzymes: induction of their properties. [9]

- **Intoxication signs:** Especially the gastrointestinal tract (GIT), the kidneys and the liver are sensitive against the EO which irritates their mucous membranes. The first intoxication symptoms are gastroenteritis with diffuse hyperemia, furthermore, headache, facial flushing, impaired vision, vertigo, incoordination and paresthesia which are caused by changes in the central nervous system (CNS). Additionally an overdose of the EO can lead to death in humans and rats. The cyto- and genotoxic effects of *C. ambrosioides* extracts are probably related to the EO. [12]

Summary:

The study and its assays attribute to the EO a strong pest control potential against DBM. It did not only show its contact and fumigant toxicity, but also its antifeedant activity, pupation inhibition as well as its concentration-depending inhibition and induction of important endogenous protective enzymes. Because of these promising properties it is imaginable that this EO can be used as an effective insecticide. Because of the irritating effect of the

EO against the GIT, the kidneys and liver of humans and the confirmed cyto- and genotoxic effects of the extracts, one can suppose that this EO could possess the same properties. [9] [12]

10. *Cymbopogon martini, C. winterianus, C citratus, Vetiveria zizanioides* (Poaceae):

Palmarosa (*C. martini*), citronella (*C. winterianus*), lemongrass (*C. citratus*) and vetiver (*V. zizanoides*) EOs are common used in the food industry as flavoring and preservative agents (beverages, chewing gimbaled products), in the cosmetic industry as perfume, in the aromatherapy as well as in the medicine because of their antimicrobial, anti-fungal, anti-protozoal and antibacterial activity (MIC = 0.05–5.0 microliter/ml). [10]
Generally the EOs are classified as GRAS (= Generally Recognized As Safe) at low concentrations, but to achieve for example the same antibacterial effects in foods, much higher concentrations are needed which are not safe for humans anymore. Possible consequences could be mutations, cariogenic effects and genetic damages. That is the reason why a few experiments were taken to evaluate the toxicity (cyto- and genotoxicity) of the EOs (palmarosa, citronella, lemongrass and vetiver) and their monoterpenoids (citral and geraniol) in human lymphocytes. [10]

The EOs show few differences in the composition especially regarding the amounts of the constituents. [10]

- ***C. martini*:** The major components are geraniol (65-85%) and greenly acetate (5-20%). [10]
- ***C. winterianus*:** Mainly consisting of geraniol (20-40%), citronellal (20–30%) and citronellol (10–15%). [10]

- ***C. citratus*:** Showes citral (50-88%) as the main constituent as well as linalool, myrcene, geraniol, geranyl acetate and camphene. [10]

Results:

The experiments were performed with human peripheral blood from healthy male volunteers. As a preparation for the tests the lymphocytes were isolated, incubated (3h at 37 degrees celsius) and washed. [10]

- **Cytotoxicity:** The cytotoxicity was evaluated by means of the trypan blue dye exclusion method and the MTT assay as already mentioned. Therefore, the EOs were tested in different concentrations. Palmarosa-, citronella-oil and geraniol in higher doses (100, 200, 500, 1000, 1500 and 2000 µg/ml), on the other hand lemongrass-EO, citral and vetiver acetate EO in lower ones (25, 50, 100, 200, 400 and 800 µg/ml). The assays resulted in a reduction of human lymphocyte-viability with the greatest activity showed by citral which reduced the cell viability to 70.7%. [10]

- **Genotoxicity:** The highest potential which means DNA damage and DNA stand breaks on human lymphocytes were detected in the lemongrass essential oil (100 µg/ml) and its major component citral (25 µg/ml). Followed by the vetiver acetate oil (>100 µg/ml) and palmarosa- as well as citronella-oil (>1000 µg/ml). Only geraniol did not show any significant genotoxic effects. [10]

- **Apoptosis/Necrosis:** Annexin V-FITC/PI staining was used for the quantification of cell death. The apoptotic and necrotic activities as usual vary between the different EOs and constituents. Citral represents again the highest effects with 43.9% cell death. The citronella (39.6%) and lemongrass EO (30.1%) activities should not be neglected either. The tail light and consequently the safest EO and component are again palmarosa EO (10.8%) and geraniol (7.2%). [10]

- **ROS (= reactive oxygen species) level in lymphocytes:** The evaluation was performed using the fluorescent DCFH-DA method. The treatment with palmarosa oil and geraniol showed no changes in the ROS level, while citronella, lemongrass oil and citral led to ROS-increase. [10]

Summary:

EOs of the genus *Cymbopogon* and *Vetiveria* are used in different areas and are useful and popular substances. Because of that it is important to know about their toxic potential. Higher doses can lead to different cell damages and health problems. After screening a few EO of the Poaceae family it can be said that oils with high amounts of geraniol could be classified as safe in comparison to these which possess citral as major component. As always the composition and concentration of the constituents are responsible if an EO is harmful and toxic or if it can be used fearlessly. [10]

11. *Alpinia zerumbet* (Zingiberaceae):

Alpinia zerumbet is one of many plants which are commonly used in folk medicine of developing countries especially for their primary health care. It is native in subtropical and tropical areas of Asia and America. Lots of great properties are attributed to the *A. zerumbet* EO such as antihypertensive, anti-nociceptive, anxiolytic, antipsychotic, antispasmodic, anti-inflammatory and antioxidant activity. Because of that it is used for the treatment of different diseases (intestinal disorders, hypertensive cardiovascular disease). [11]
The EO of the leaves consists of monoterpenes (98.2%) and sesquiterpenes (1.8%) including 1,8-cineol (22.4%), terpinen-4-ol (17.3%), γ-terpinene (11.4%) and sabinene (9.9%) as its major constituents. [11]

Different important properties of the EO were tested *in vivo* (a single oral dose was given to male and female Swiss mice) and *in vitro* (on human leucocytes). Probably the most interesting thing was the huge concentration-dependent effects and how the application-time can change the EOs' activities. [11]

Results:

- **Cytotoxic, genotoxic and mutagenic effects (*in vitro*):** At the concentration of 500 µg/ml and above the EO showed different toxic effects as the decrease of the viability, the proliferation rate of the leucocytes and of the cytokinesis-block proliferation, as well as DNA damage, chromosome and chromatid breaks and increase of cytogenetic abnormalities. Nevertheless, lower concentrations of the EO showed as a post-treatment (after H_2O_2) great effects like DNA repair, cell viability increase, cell apoptosis and anti-mutagenic effects. But it caused opposite effects when used as pre-treatment. [11]

- **Genotoxic and mutagenic effects (in vivo):** No toxic effects could be noted in mice which were treated with non-toxic doses (concentrations to 400 µg/ml) of the *A. zerumbet* EO. Also differences between the sexes were not existent. [11]

- **Antioxidant activity:** With increasing concentrations the antioxidant activity of the EO against DPPH raised too. In fact the effect of a 300 µg/ml dose (= more than 50% reduction of the radical) is similar to the antioxidant activity of ascorbic acid (dose of 150 µM), one of the greatest antioxidants. It did not lead to intracellular GSH (= glutathione) level reduction and the increase in oxidative stress markers. It can counteract the effects caused by H_2O_2 when it is given as a co-treatment which means prevention of the GSH level increase, the ROS formation, the lipid per-oxidation and the oxidation of nucleotide bases. This means that the EO protects the leucocytes of the harmful effects (cytotoxicity)

caused by H_2O_2. Given as a pre- or post-H_2O_2 treatment these protective effects could not be noted. On the other hand a 500 µg/ml dose did not only operate non-protective, it also increases the cytotoxicity of H_2O_2. [11]

Summary:

A. zerumbet EO is one of few EOs which causes exactly opposite effects depending on the concentration. As it is common that the doses are responsible for the activity of a plants preparation, herein it can operate either protectively against cytotoxicity or it is cytotoxic itself. Because of that using the right dose of this EO is the major priority, otherwise bad consequences as cancer development can be the result. [11]

12. *Curcuma zedoaria* (Zingiberaceae):

Curcuma zedoaria, as known as white turmeric, is a common plant in the indonesian medicine used for the treatment menstrual disorders, dyspepsia, vomiting and cancer. [13]

After isolating the EO from fresh rhizomes, by steam-distillation, in this case and identifying the constituents, camphor (49.5%) has appeared to be the main component and shows different important properties as analgesic, antipyretic, decongestant and expectorant activity and is often used for topical medicine, but also some of the other constituents exhibit meaningful effects as furanodiene (3.6%) and furanodienone (3.5%) operate anti-inflammatory or 1,8-cineole (3.4%) shows antibacterial activity against *S. aureus*, *E. coli* and *Pseudomonas aeruginosa*. Furthermore, one can find isobornyl alcohol (12.7%) and borneol (4.2%) as important parts of the composition. [13]

It is interesting that the EOs from *C. zedoaria* rhizomes from India, China or Japan are different compounded as the one from Indonesia. For example curzerenone, 1,8-cineole and germacrene are the major components of the Indian species or furanogermenone, curcumenol and dehydrocurdione of the Chinese and Japanese one. That implies that the EOs properties depend on the plants geographical origin. [13]

Results:

- **Acute toxicity:** The smallest measured LD_{50} value of the EO was 3.25 ppm which means that it can be classified as anti-tumor-active (values <40 ppm). Champor, furanodiene and furanodienone have the most toxic effect against *A. salina*. [13]

Summary:

Once again it was noticed that the properties, effects and toxicity levels of EOs of the same plant can vary strongly depending on its origin and the composition, especially considering the amount of the particular constituents. [13]

13. *Echinophora orientalis* (Apiaceae):

The *Echinophora* genus contains of 10 species and grows in the Mediterran and Middle East regions. The analysis of the EOs' seeds, shoots and roots showed the differences between the composition of these plant parts. Six constituents (α-pinene, myrcene, p-cymene, I-limonene, p-cymen-8-ol and 1,7-octadiene 3,6-dimethylene) can be found in all three parts, but the amount varies heavily. [14]

On closer consideration it is easy to see how different parts of one plant can vary from one another and that it is important to know which EO is used. Different compounds mean different biological activities. [14]

- **Volatile oil of the seeds:** The biggest difference between the EOs of the seed and the shoots and roots, is that they mainly consist of sesquiterpenes (56.7%) but as well as monoterpenes (10.1%) and non-terpenoid volatile compounds (7.2%). The main amounts represent spathulenol (10.5%), carotol (9.5%), bicyclogermacrene (4.5%) and germacrene D (3.7%). Additionally, it is important to say that a high ratio of the constituents is oxygenated. [14]

- **The shoots:** With 72.6% the EO of the shoots mainly consists of monoterpenes. Of the total compound 77.4% are non-oxygenated hydrocarbons. Mentionable EO compounds are myrcene (20.9 %), p-cymene (11.5 %), α-pinene (7.7 %), α-phellandrene (6.0 %) and β-phellandrene (3.3 %). [14]

- **The roots:** The volatile oil of the roots is mainly composed of non-terpenoid compounds (60.8%) and monoterpenes (36.8%). Of which 67.6% are oxygenated hydrocarbons. Important constituents are myristcin (52.9 %), terpinolene (24.4 %), falcarinol (4.8 %) and myrcene (3,0 %). [14]

Various activities are attributed to the EO including antibacterial, anti-fungal, antioxidant, anticancer activity as well as wound healing properties.
The DPPH assay showed that the EO possesses weak to moderate free-radical-scavenging properties. [14]
Unfortunately the insecticidal test against Tribolium castaneum did not provide any effects, but the brine shrimp lethality assay showed a general toxicity with LC_{50} values = 0.0026, 0.0201 and 0.0002 mg/ml. [14]

Summary:

After the identification of the compounds and the analysis the roots volatile oil of *E. orientalis* showed the most effective bioactivity as well as the highest antioxidant properties, but also the most efficient general toxicity. [14]

14. *Eucalyptus* species (Myrtaceae):

The *Eucalyptus* genus includes 900 species. The main component of the EOs is eucalyptol (= 1,8-cineole), a cyclic monoterpene ether which can also be found in other plants of the genera *Achillea*, *Cinnamomun*, *Laurus*, *Lavandula*, *Melaleuca*, *Mentha*, *Rosmarinus* and *Salvia*. [15]
Some cases of human poisoning after the intake of *Eucalyptus* EO are known, as well as its broad antibacterial and anti fungal activity. Furthermore, antioxidant, anti-inflammatory, antiseptic and vasorelaxant effects in rats are reported. [15]

Because of the increasing resistance of microorganisms against synthetic acquired repellents, natural products as EOs are used more often in this sense. The reported insecticidal activity of eucalyptol is a great base to test how effective the substance really is. It shows stronger lethal effects than other monoterpenes. [15]

In this case the effect of the EOs on locomotor activity, knock-down, and repellence as well as the caused visible intoxication-signs against the nymphs of *Triatoma infestans* and *Rhodnius prolixus*, the causer of the Chagas disease, were tested. [15]

Results:

- **Visible intoxication symptoms:** Common signs are leg paralysis, incoordination (= abnormal movements of proboscis and antennae), hyperactivity, other symptoms similar to toxic effects on the nervous system and abdominal distention. [15]

- **Effect on locomotor activity:** The EO induces an increase in locomotor activity in both microorganisms, nevertheless the effect was not as mentionable as expected. [15]

- **Knock-down analysis:** This test showed the best results. The nymphs were not only kocked-down by the eucalyptol, they also were not able to recover after the expose to the highest applied concentration of the oil. [15]

Another example which shows the toxic effects against insects is the EO (using the leaves) of the species ***E. cinerea***. It mainly consists of 1,8-cineole (88.5%), α-pinene (2%) and α-terpineol (9%), furthermore β-pinene, β-terpineol, geranial and camphene. [16]
Musca domestica adults, better known as the houseflies, were taken for the evaluation of the EOs' toxic activity. As the flies are carrier of pathologic microorganisms and different illnesses and the chemical pest control methods are also harmful for humans, a natural insecticide would be a great and more healthy alternative for this use. [16]

After exposing the flies to the EO, 1,8-cineole or acetone (control substance) mortality was evaluated 15-30 minutes later, thereby the most absorbed component with the highest lethality rate was 1,8-cineole (74%; LD_{50} = 3.3 mg/dm³) followed by α-terpineol (24.7%; LD_{50} = 36.8 mg/dm³), a new identified constituent 2,3-dehydro-1,8-cineole (1.2%) and α-pinene (0.1%; LD_{50} =11.5 mg/dm³). α-Terpineol and 2,3-dehydro-1,8-cineole are probably metabolites of 1,8-cineole and the result of the reaction between the component and the P450 oxidative system, which would explain their appearance in the flies' organisms. Another test was taken to confirm this statement: The EO and PBO (= piperonyl butoxide), a P450 inhibitor, was additionally given to the flies and the results are incredible. Not only the LD_{50} value of 1,8-cineole was increased from 3.3 to 1.6 mg/dm³ also its concentration was higher (90,8%) and the amount of the metabolites was lower which means that the oil is more toxic when the P450 oxidation system is inhibited. Therefore, it seems that the metabolites are important for the detoxification of eucalyptol. [16]

As a matter of fact, 1,8-cineole acts in higher doses as a P450-inhibitor-like-compound because of its competition about the oxidation with other xenobiotics. In combination with deltamethrin (= insecticidal substance) for example it leads to the same lethal effect but with lower doses of the insecticide. [16]

Summary:

Monoterpenes like eucalyptol are a great alternative to highly toxic synthetic repellents. They show potent insecticidal effects, humans are not as effected by their toxicity as by the toxic activity of non-natural products and there are no reported resistant microorganisms against them. Although lots of positive results were brought out, eucalyptol cannot achieve the strong repellent effects as chemical insecticides. Nevertheless, it showed great potential in this use especially against resistant houseflies which is the reason enough for more studies and experiments with *Eucalyptus* EOs in this matter. [15]

15. *Garcinia mangostana* (Clusiaceae):

Garcinia mangostana is an evergreen tree with typical taste and fragrance, native in tropical areas of Thailand, Australia and some South-east Asian countries. In Asian folk medicine it is commonly used because of its astringent, antioxidant, anti-inflammatory, antibacterial, anti fungal, antiviral, anti-tumoral and anti-cancer activities for the treatment of abdominal pain, diarrhea, dysentery, enteritis, infected wounds and chronic ulcer. [17]

The leaf (64 constituents) and stem-bark (101 constituents) EOs consist mostly of sesquiterpene hydrocarbons (82.9-87.9 %), oxygenated sesquiterpenes (6.2 -9.1 %) and diterpenes (1.2-1.9 %). [17]
The EOs differ in their composition and concentration of the major components. [17]

- **Leaf EO:** Consisting of β-caryophyllene (21.1 %), β-elemene (9.7%), germacrene D (7.9 %), α-selinene (7.4 %), α-humelene (7.3%) and β-selinene (7.1%). [17]

- **Stem-bark EO:** Herein β-caryophyllene (17.3%) is identified as the major component as well. Following by β-selinene (10.7%), trans-α-bergamotene (7.5%), α-selinene (6.4%) and α-humelene (6.4%). [17]

The anti-inflammatory, local anesthetic, anti-fungal and cytotoxic properties as well as the apoptosis inducing activity of the EO are attributed to β-caryophyllene. [17]

Results:

- **Acute toxicity:** The LC_{50} values after 24h were 1.70 for the leaf and 5.15 µg/ml for the stem-bark EO and is therefore classified as highly toxic. [17]

- **Antibacterial activity:** Both EOs possess antibacterial activity. Nevertheless, the highest MIC level and inhibition zone (48.2 mm) against different bacteria like *S. epidermidis*, *S. aureus*, *E. coli*, *Proteus* species and *Bacillus subtilis* showed the essential oil of the *G. mangostana* leaves. [17]

Summary:

The toxicity and antibacterial effects show the EOs' potential as useful pharmaceuticals in the cancer therapy and as strong repellents especially against antibiotic-resistant microorganisms. More studies are needed to confirm the use oft the EOs in this range. [17]

16. *Lippia alba* (Verbenaceae):

Lippia alba is another plant whose EO cannot only be used as a medical agent but as well as an insecticide. This plant appears in different chemotypes which are characterized in the carvone-chemotype and the citral-chemotype. As expected, these chemotypes exhibit a different composition pattern and accordingly various properties. [18]

- **Carvone-chemotype:** As the name tells this chemotype possesses carvone, an unsaturated monoterpenoid ketone, as its major constituent. The amount of carvone comes to 52.9-63.5% depending on the chemotype. [18]

- **Citral-chemotype:** The main component here is citral, a mixture of geranial (44.2-46.3%) and neral (31.1-33.5%) which are double-bond-isomeric acyclic monoterpene aldehydes. [18]

Results:

Acute Toxicity:

The acute toxicity of the citral-chemotype was evaluated by treating mice with different doses of the EO. Concentrations of 1000mg/kg and higher showed clear toxic effects, such as hypotonia, dyspnoea, convulsions, inflammation in liver as well as a decrease in locomotion, motor skills and muscle strength and neurotoxic effects. The calculated LD_{50} value was 2500 mg/kg. [19]

Toxicity assays: Additional toxicity bioassays were performed against two insects *Sitophilus zeamais and Tribolium castaneum*. Not only the LD_{50} but also the LT_{50} (lethal time to kill 50% of the insects) values were tested. In general, the isolated monoterpenes carvone and citral showed higher toxicity

then the EOs, the carvone-chemotype operated more toxic and *S. zeamais* reacted more sensitive than *T. castaneum*. [18]

The LD_{50} and LT_{50} values at a dosage of 10 µL/mL were as follows:

- *S. zeamais*: 15.2-16.7 µL/mL for the EO of the carvone-chemotype (LT_{50} = 8.2h) and 54.6-70.8 µL/mL of the citral-chemotype (LT_{50} = 13.7h) . Furthermore, the values of the isolated monoterpenes are carvone 6.9 µL/mL and citral 19.8 µL/mL. [18]
- *T. castaneum*: Carvone-chemotype: 19.5-28.7 µL/mL (LD_{50}) and 15h (LT_{50}), citral-chemotype: 67.2-107.8 µL/mL (LD_{50}) and 62.7h (LT_{50}), isolated carvone: 8.8 µL/mL and isolated citral: 18.1 µL/mL. [18]

Repellency assay: The EOs and the isolated monoterpenes showed no repellency effects on *S. zeamais*, but *T. castaneum* was susceptible to the EOs and the monoterpenes equally. [18]

Summary:

EOs from different plant-chemotypes are always difficult to evaluate because of the differences in their composition which lead to various biological and biochemical properties. The *L. alba* EO is another example for that. In fact it showed high toxic effects against both insects, *S. zeamais and T. castaneum*, nevertheless the carvone-chemotype possesses more potential toxic effects. As the carvone-chemotype is considered to be the most toxic, one could assume that the evaluated toxic effects of the citral-chemotype in mice could be attributed to the carvone-chemoype too. To confirm this statement more studies have to be performed. [18] [19]

17. *Litsea elliptica* (Lauraceae):

Litsea elliptica is a tropical tree native in South-east Asia. Different preparations of the plant are used in the folk medicine for the treatment of headache, fever, stomach ulcer and as insecticides (mosquito control). [20]
The EO consists of sesquiterpenes (76.3%), sesquiterpene alcohols (10.1%) and aldehydes (10.8%), including δ-cadinene (13.2%) as one of the major components. [20] [21]
For the evaluation of the EOs' acute and subacute toxicity female Sprague-Dawley rats were used. [20]

Results:

- **Acute toxicity:** The analysis of the acute toxicity took place 14 days after the treatment (single dose administration of 500, 1000, 2000, 2500, 3000, or 4000 mg/kg). Toxicity signs like hypoactivity, lacrimation, and piloerection were determined at doses of 1000 mg/kg and more and body weight decrease as well as mortality from 2500 mg/kg onwards. In the group which received the highest dose just a few animals survived. Consequently, the calculated LD_{50} value is 3488.86 mg/kg. Functional disorders of the autonomic nervous system and the central nervous system are probably the reason for those symptoms which are similar to the symptoms caused by organophosphate insecticides like malathion and diazinon. [20]

- **Subacute toxicity:** The only changes noted after a four week treatment with the EO (doses of 125, 250, and 500 mg/kg) were an increase of the haemoglobin concentration and MCV (mean cell volume), MCH (mean cell hemoglobin), MCHC (= mean cell hemoglobin concentration) levels decrease but the values were still in the normal range. Furthermore, RBC (red blood cell) differences as to the change of their shape into an echinocytic form and the decrease of their membrane phospholipids and

cholesterol (but no membrane damage) were notable. Deaths, toxicity signs or organ damages (kidneys, liver, pancreas) were not reported and also the pathological examination was without any interesting result. [20]

Summary:

As the EO of *L. elliptica* is similar effective to well known insecticides, it is a real alternative to be used in the insect control. Especially because of its less harmful effect on mammals when given in appropriate doses compared with chemical repellents. [20]

18. *Mentha* species (Lamiaceae):

Menthol is the major component of all *Mentha* species. It is classified as safe and possesses cooling, muscle relaxing and analgesic effects. Besides the well known *Mentha piperita*, there are less popular *Mentha* species as the Chinese one, *Mentha haplocalyx.* In Asia it is very popular because of its great effects treating nerve-center-, breath-, procreation- and digestive-system-disorders. [22] [23]

As a potential natural repellent and insecticide the EO of *M. haplocalyx* was tested against the *Lasioderma serricorne* adults to evaluate its toxicity and antimicrobial activity. According to further reports the EO operates more effective than the commonly used synthetical pesticides like organophosphorus antimicrobials. [22]

The analysis of its composition showed 23 constituents and menthol (59.7%), menthyl acetate (7.8%), limonene (6.9%) and menthone (4.4%) are identified as the major components. [22]

Results:

- **Contact Toxicity:** The LD_{50} values between the EO itself and its constituents vary as expected. The highest effects showed menthyl acetate with 5.96 µg/adult. Almost 3 times higher and therefore the lowest contact toxicity possessed the oil itself with 16.5 µg/adult. The value of menthol is comparable with menthyl acetate-result and the one of limonene with the oils yield. [22]

- **Repellent activity:** The repellent effects depend on the used concentration and on the exposure-time. Herein menthol showed the strongest repellent property (after 2h and 4h treatment), it possessed even greater effects than the pesticide DEET (diethyltoluamid). [22]

- **Intoxication signs:** As mentioned before *Mentha* species are considered as safe, but a constant exposure as well as hight doses (>1g/kg body weight) can have harmful consequences, such as hypersensitivity, contact dermatitis, abdominal pain, acid reflux disease, perianal burning, bradycardia and muscle tremor, but also vertigo, dizziness, nystagmus, ataxia, hallucinations, lethargy and coma. [23]

Summary:

For a certain statement about the toxicity and repellent/insecticide effects of the *M. haplocalyx* EO and its constituents and consequently their possible usage, more studies have to be performed. Ecological prevention and control of storage pest could be a potential application area. For a safe usage of the EOs of different *Mentha* species, it is important not to use high doses and to delimit the treatments duration. [22] [23]

19. *Rosmarinus officinalis* (Lamiaceae):

The EO of *Rosmarinus officinalis* is known in the traditional medicine for its useful properties. It operates stimulating, astringent, carminative and anti-inflammatory as well as antimicrobial. This is the reason why its effects on *Sitophilus oryzae* and *Oryzaephilus surinamensis* have been studied. These insects infest especially important foods like rice, wheat, dry fruits and grain oats which is the reason why natural and safe alternatives to the harmful chemical insecticides have to be found. [24]

The EO's identification yielded 50 compounds with 2-methoxy-3-(2-propenyl)-phenol (28.2%), 1,8-cineole (25.1%) and camphor (14.6%) as the major constituents. [24]

Results:

- **Fumigant toxicity:** The fumigant toxicity assay showed that both *Sitophilus oryzae* (LD_{50} = 0.057 µl/ml air) and *Oryzaephilus surinamensis* (LD_{50} = 0.039 µl/ml air) were highly susceptible to the *R. officinalis* EO. 100% mortality was achieved with a concentration of 0.15 µl/ml air (maximum tested dose) during an exposure of 36h for *O. surinamensis* and 72h for *S. oryzae* which confirms the fact that the effect depends on the concentration and the exposure-time. [24]

- **Acetylcholine-esterase activity:** AChE was inhibited by all tested concentrations of the EO with the maximum effects at the dosage of 0.15 µl/ml (40.42%-44.39% inhibition). Thereby, *O. surinamensis* reacts more sensitively to the EO. [24]

- **Superoxide-dismutase (SOD) activity:** SOD is an important antioxidant which catalyzes the toxic superoxide into oxygen and hydrogen peroxide. The exposure of the insects to the EO showed in both a high increase of the SOD activity at the maximum tested concentration. [24]

- **Catalase (CAT) activity:** CAT is an antioxidant enzyme which splits hydrogen peroxide into water and oxygen. The results after the EO-treatment correlate with the results of SOD. [24]

- **Glutathione (GSH) level:** GSH is an important agent for the maintenance of the cellular redox homeostasis. Changes of the GSH level can lead to a stressful and toxic state to the cell. The toxic effects of the EO could be a result of this oxidative imbalance. *S. oryzae* showed at the maximum dosage after 48h a GSH level a decrease of 7.02% in comparison to the control and *O. surinamensis* after 12h a reduction of 24.76%. [24]

- **Toxicity symptoms:** In high doses the EO can irritate and damage the stomach, intestinal, the kidneys as well as the skin and cause allergic contact dermatitis. Furthermore, it operates as an abortive and convulsant agent. Concentrations of the EO which lead to these consequences, can be classified as toxic. [25]

Summary:

The EO of *R. officinalis* showed great potential in the pest control against *Sitophilus oryzae* and *Oryzaephilus surinamensis*. It is not only very effective but acts also with consideration regarding the germination. Because of that more investigations should be performed to confirm the EOs' potential as an insecticide. The toxicity signs can only be noticed in high concentrations. [24] [25]

20. *Vitellaria paradoxa* (Sapotaceae):

The *Vitellaria* genus is local in Africa and different species are well known and commonly used in the folk medicine. One of these species is *V. paradoxa.* Its EO - which is mostly processed to Shea butter oil - possesses different interesting properties and is used in the treatment of various ailments and diseases as rheumatism, rashes in children, dermatitis, irritation, sunburn, gastric problems, headache, diarrhoea, dysentery and also as an anti-inflammatory agent. Furthermore, it shows antimicrobial activity (against *S. lutea* and *S. aureus*) and can be supportive and promoting during child birth. [26]

The identification of the leaves and stem-bark *V. paradoxa* EOs showed different composition patterns, especially in the quantity of the constituents. Nevertheless, the major compound in both oils is linalool. [26]

But now a more exact discussion about the identification of the two EOs is necessary:

- **EO of the leaves:** The substance groups which represent the leaves EO are non-terpene derivatives (38.7%), oxygenated monoterpenes (19.7%), apocarotenoids (13.6%), sesquiterpene hydrocarbons (7.2%), oxygenated sesquiterpenes (5.7%), monoterpene hydrocarbons (3.9%) and phenylpropanoids (1.3%). Linalool (12.2%), β-ionone (8.4%), methyl salicylate (7.0 %), nonanal (6.9%) and 1-nonanol (5.8%) are the most important components. [26]

- **EO of the stem-bark:** The differences to the EO of the leaves can be discerned very well because the composition-quantity of the substance groups vary strongly. More than the half of the oil consists of non-terpene derivatives (51.6%), followed by oxygenated monoterpenes

(16.6 %), monoterpene hydrocarbons (14.8 %), apocarotenoids (5.2 %), sesquiterpene hydrocarbons (3.7%) and phenylpropanoids (3.2 %). The identified major constituents are linalool (15.0%), limonene (14.8%), n-decane (14.4%), pseudocumene (8.6%), mesitylene (8.1%), nonanal (5.5%) and geranylacetone (5.2%). [26]

Results:

- **Toxicity assay:** The brine shrimp test showed LD_{50} values of 171.240 µg/ml for the leaves EO and 160.098 µg/ml for the stem-bark EO a moderate toxicity. [26]

Summary:

As the brine shrimp test showed that the EOs of *V. paradoxa* operate toxic and that the compounds of the oil are medically active and in the African folk medicine can be used against different diseases, it would be interesting as well as useful to perform more studies about *V. paradoxa*. Because of the lack of information about the toxicity effects of the EO in humans data about the toxicity as well as the properties of the plant are necessary to evaluate if the EO can be used as a pharmaceutical agent or if it can cause damages. [26]

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